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Suppressed antibody and interleukin-6 responses to acute pyelonephritis in pregnancy

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Suppressed antibody and interleukin-6 responses to acute pyelonephritis in pregnancy. This study examined the effect of pregnancy on the host response to acute pyelonephritis. Urine and serum samples were obtained at the time of diagnosis and after two weeks, from non-pregnant and pregnant women with acute pyelonephritis. The samples were analyzed for interleukin-6 (IL-6) and specific antibody activity to antigens extracted from the *Escherichia coli* strain infecting each patient. The host response to infection was further quantitated as fever, C-reactive protein, and renal concentrating capacity. Acute pyelonephritis in non-pregnant and pregnant women was accompanied by a significant serum and urine antibody response. The serum antibody response was significantly lower in the pregnant group. The IL-6 levels in serum and urine at diagnosis were significantly higher in the non-pregnant compared to the pregnant women. These results demonstrate that the immunosuppression of pregnancy includes the mucosal IL-6 and specific antibody responses to acute pyelonephritis caused by *E.coli*.

Urinary tract infections [UTI] elicit a host response which includes the production of specific antibodies and the secretion of cytokines. While uninfected urine contains immunoglobulin and immunoglobulin fragments [1–3], it has little specific antibody activity against the bacterial pathogens which commonly cause UTI [4, 5]. Bacterial infections, on the other hand, stimulate both local and systemic immune responses [5–8]. The urinary antibody response to *Escherichia coli* is dominated by secretory IgA (sIgA) [2–8], but the levels of monomeric IgA and IgG in urine may also rise during acute infection [9]. *E.coli* also activate mucosal and systemic cytokine responses. Interleukin-6 (IL-6) is secreted into the urine of patients with acute pyelonephritis and asymptomatic bacteriuria (ABU) [10, 11]. The serum IL-6 levels are elevated in patients with acute pyelonephritis, but not in those with ABU [10, 11].

Pregnant women run an increased risk of developing acute pyelonephritis, suggesting an impaired resistance to infection [12–16]. Pregnancy is accompanied by suppression of the host's immune response, such as reduced cytotoxic and increased suppressor T-cell activity [17–22]. The *in vitro* response of peripheral blood lymphocytes to concanavalin-A is impaired, but the responses to pokeweed mitogen and phythemagglutinin are intact [17–19]. The studies on immunoglobulin levels and antibody activity are partly conflicting. Bisset et al found reduced circulating IgG but intact IgM levels in pregnant women [20]. Kumar, Madden and Nankervis saw no change in antibodies to cytomegalo- and herpes simplex virus but a decreased cellular immune response to the same agents [21].

A suppressed immune response might contribute to the susceptibility of pregnant women to symptomatic UTI. The influence of pregnancy on the mucosal immune response has not been extensively studied. The aim of the present study was to examine the influence of pregnancy on the IL-6 and specific antibody responses to acute pyelonephritis caused by *E. coli*.

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Methods

Patients

The non-pregnant and pregnant women participated in a prospective study of acute pyelonephritis [23, 24]. Enrollment took place at the Infectious Diseases Clinic, Göteborg, Sweden or at either of the two maternity hospitals serving the study population. Diagnosis, treatment and follow-up were performed according to a standardized protocol [24].

Diagnostic criteria and treatment

All patients had significant bacteriuria defined as $\geq 10^5$ colony forming units of one bacterial strain per ml of freshly voided midstream urine. The clinical diagnosis of acute pyelonephritis was made in patients who had fever $\geq 38.0°C$ with costo-vertebral angle tenderness and/or loin pain. Dysuria, frequency of urination, nausea, vomiting and chills were other frequent symptoms. The patients diagnosed as having acute pyelonephritis were tested for C-reactive protein (CRP) in the serum and had their renal concentrating capacity determined [23]. The serum CRP concentration in mg/liter was measured by radial immunodiffusion at the time of diagnosis. Levels greater than 25 mg/liter were considered elevated. The maximal renal concentrating capacity was determined as the osmolarity (mOsm/kg) in two consecutive urine samples obtained after 16 hours of fluid deprivation. The higher value of the two was chosen. Each patient received a two week course of antimicrobial therapy. The choice of drug was based on the antibiotic sensitivity pattern of the infecting strain.

Sample collection and culture

Serum and urine specimens were obtained at the start of treatment and two weeks later. All samples were stored at −20°C prior to analysis. Urine and blood samples for culture were obtained prior to treatment. Isolated bacteria were identified to the species level by standard bacteriological techniques and stored as deep agar stabs. Extensive information on the O:K:H serotypes and virulence factors of the isolated *E.coli* strains have been presented elsewhere [24, 25].

Determination of specific antibody activity

The antibody response was analyzed in patients with infections caused by *E.coli*, who had serum and urine samples taken at diagnosis and two weeks later. This subsample consisted of 48 non-pregnant women (median age 52 years, range 19 to 80) and 19 pregnant women (median age 26 years, range 20 to 36). One pregnant and 14 non-pregnant women had positive blood cultures.

Antigen extracts were prepared from the *E.coli* strain infecting each patient, as previously described [26]. Briefly, each strain was cultured overnight on solid medium and harvested into phosphate-buffered saline (PBS, pH 7.2, 0.1 mM). The number of bacterial cells in the suspension was determined as the optical density at 595 nm. The bacterial suspension was boiled for two hours in a water bath. The bacterial fragments were sedimented by centrifugation at 2000 × g for 20 minutes, and the supernatants used as antigens. The concentration of the antigen extract was adjusted by dilution in PBS to correspond to a bacterial concentration of 2×10^9 bacteria/ml. This procedure was previously shown to yield comparable amounts of heat-stable antigens from different *E.coli* strains [26]. A pool of *E.coli* antigen extracts was used as a control. These extracts were prepared from *E.coli* strains of the serogroups which commonly cause UTI (O1, O2, O4, O6, O7, O8, O16, O18, O25 and O75) [27].

Microtitre plates (Nunc, Copenhagen, Denmark) were coated with 0.1 ml of the control pool of antigens or the heat-stable antigen extracts from the individual *E. coli* strains. The coating procedure was previously shown to result in an antigen excess sufficient for the detection of antibodies to different antigenic specificities within the extracts [26]. The plates were incubated for four hours at 37°C and overnight at +4°C. Unbound antigen was removed by repeated washing with PBS containing 0.05% Tween 20 (PBS-Tween).

Urine and serum samples were diluted in PBS-Tween. Urine samples were analyzed at a 1:2 dilution. Serum samples were diluted 1:100, 1:1000 and 1:10000. The samples were added in duplicate (0.1 ml/well) and incubated for four hours at 37°C. Unbound antibody was removed by repeated washing with PBS-Tween. The bound antibody was detected using alkaline phosphatase conjugated goat anti-human IgG, IgA and IgM (Orion, Helsinki, Finland). The antibody conjugates were diluted according to the manufacturer's instructions and used at 0.1 ml per well. After incubation at room temperature for 16 hours, unbound conjugate was removed by washing with PBS-Tween. The substrate (p-nitrophenyl phosphate) was added to each well (100 μl of a 1 mg/ml solution) and incubated at 37°C. The antibody activity was given as the optical density at 405 nm after 100 minutes of incubation with the substrate.

Each sample dilution was run in duplicate in: (i) uncoated microtitre wells; (ii) wells coated with the antigen extract from the infecting *E.coli* strain; and, (iii) wells containing the control antigen pool. A serum sample serving as a positive control was run in wells coated with the control antigen pool. This serum sample had high anti-*E.coli* antibody activity and was obtained from a patient who had a history of multiple episodes of acute pyelonephritis. The antibody activity in the serum samples from each patient was expressed as a percentage of the antibody activity of the serum control. The antibody activity of the urine samples was given as the optical density × 10^3.

IL-6 determination in urine and serum

Samples (29 urines and 28 serum samples) for IL-6 measurements were available from 29 non-pregnant women with a median age of 33 years (range 19 to 67). Samples (18 urines and 12 serum samples) were available from 18 pregnant women with a median age of 24 years (range 20 to 36). One pregnant and seven non-pregnant women had positive blood cultures.

The cell line B13.29 which is dependent on IL-6 for its growth has been described [28]. For IL-6 determinations the more sensitive subclone B9 was used [29]. The B9 cells were harvested from the tissue culture flasks, and 5000 cells per well were seeded into microtiter plates (Nunc, Roskilde, Denmark) containing dilutions of sample or recombinant human IL-6 (as a standard) and Iscoves Modified Dulbeccos Medium supplemented with 50 mM 2-mercapto-ethanol, 5% fetal calf serum (Sera-Lab, Sussex, UK) and gentamicin (0.1 mg/ml), in a total volume of 0.2 ml. [^{3}H]-thymidine was added between 68 and 72 hours of culture, and the incorporated radioactivity was measured and compared to a standard curve. The standard curve was prepared using stock recombinant human IL-6 (8000 U/ml; one unit = the concentration of IL-6 required for half maximal thymidine incorporation). The activities of each sample are given as U/ml when compared to the standard curve.

Statistical methods

Differences between groups were evaluated using the Mann-Whitney U test. Pairwise comparisons were performed by Wilcoxon's signed rank test. Two-tailed tests were used and $P < 0.05$ was considered as statistically significant. Correlations between parameters were tested using linear regression.

Results

Serum antibody response to acute pyelonephritis in non-pregnant and pregnant women

Paired serum samples were obtained from 48 non-pregnant women at the time of diagnosis and after two weeks. The IgG, IgA, and IgM antibody activities are shown for each patient in Figure 1. The median and range values of IgG, IgA, and IgM at diagnosis and after two weeks are shown in Table 1. We compared the values at the time of diagnosis with those obtained at the two week follow-up in the individual patients using the Wilcoxon signed rank test. There was a significant increase in the serum IgG, IgA and IgM antibody activity in the non-pregnant group (Table 1).

Paired serum samples were obtained from 19 pregnant women at the time of diagnosis and after two weeks. The IgG, IgA and IgM antibody activities for the individual patients are

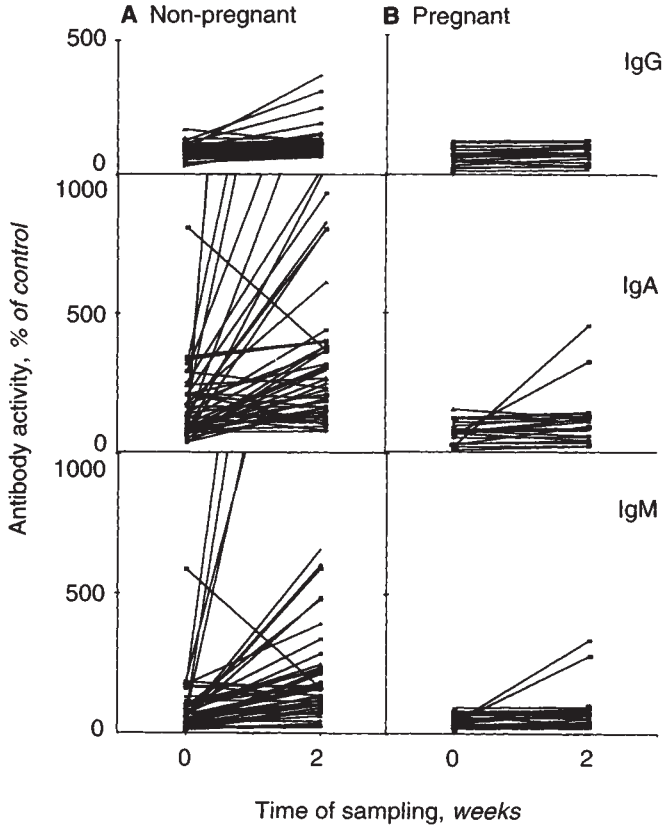

Fig. 1. *Serum antibody activity (IgG, IgA, IgM) at the time of diagnosis of acute pyelonephritis and at the 2 week follow-up.* Samples from the non-pregnant (**A**) and pregnant (**B**) women are shown separately. Samples obtained from each patient are connected with a solid line.

shown Figure 1. The median and range IgG, IgA and IgM antibody activities at diagnosis and after two weeks are shown in Table 1. The antibody activity at the time of diagnosis and after two weeks were compared using the Wilcoxon signed rank test. There was a significant increase in IgG, IgA and IgM antibody titers between diagnosis and the two week follow-up (Table 1).

The magnitude of the antibody responses was compared between the pregnant and non-pregnant groups at the time of diagnosis and two weeks later (Table 2). The net increase in antibody activity was significantly higher in the non-pregnant than in the pregnant group, as determined by the Mann-Whitney U test. There was a significant difference in serum antibody activity between the non-pregnant and pregnant groups at time of diagnosis (IgG, $P = 0.011$; and IgA, $P = 0.003$) and at the two week follow-up (IgG, $P = 0.003$; IgA, $P = 0.003$; and IgM, $P = 0.003$).

The ages ranged from 19 to 80 years in the non-pregnant group and from 20 to 36 years in the pregnant group. To exclude the confounding influence of age variation in the analysis of the antibody responses, we compared a subset of 13 non-pregnant women (19 to 37 years) to the 19 pregnant women (20 to 36 years) using the Mann-Whitney U test. The differences in antibody responses between the age-matched non-pregnant and pregnant groups were maintained (Table 2).

Table 1. Antibody activity in serum and urine at the time of diagnosis and two weeks later

Antibody	Antibody activity (median and range)		
	At diagnosis	After 2 weeks	P[a]
Serum[b]			
Non-pregnant			
IgG	84.5 (29–166)	107 (63–369)	0.001
IgA	127 (36–809)	298.5 (78–6897)	0.001
IgM	57.5 (9–558)	159.5 (19–3786)	0.001
Pregnant			
IgG	64 (22–116)	75 (29–120)	0.005
IgA	67 (5–153)	114 (21–452)	0.019
IgM	37 (19–77)	65 (24–335)	0.003
Urine[c]			
Non-pregnant			
IgG	126 (0–1299)	78.5 (0–1216)	0.001
IgA	54.5 (9–838)	50 (0–1966)	0.001
Pregnant			
IgG	31 (0–210)	56 (0–250)	0.043
IgA	35 (0–149)	54 (0–304)	NS

[a] P value for the difference in paired samples in individual patients at the two dates (Wilcoxon signed rank test; significance <0.05). NS is not significant.
[b] Serum antibody activity given in percent of the control.
[c] Urinary antibody activity given as the absorbance at 405 nm after 100 minutes incubation with substrate.

Table 2. Change in antibody activity in serum and urine of non-pregnant and pregnant women with acute pyelonephritis

Antibody[b]	Change in antibody activity[a] (median, range)		
	Non-pregnant	Pregnant	P[c]
All patients	(N = 48)	(N = 19)	
Serum			
IgG	20 (−46–277)	10 (−16–45)	0.003
IgA	133 (−442–6833)	21 (−31–432)	0.003
IgM	87 (−424–3630)	17 (−22–298)	0.003
Urine			
IgG	−22 (−919–1050)	27.5 (−78–127)	NS
IgA	−9 (−681–1925)	15 (−39–234)	NS
Age-matched subgroup	(N = 13)	(N = 19)	
Serum			
IgG	13 (−7–277)	10 (−16–45)	0.003
IgA	90 (−442–2390)	21 (−31–432)	0.003
IgM	87 (−424–3630)	17 (−22–298)	0.003
Urine			
IgG	−22 (−408–97)	27.5 (−78–127)	NS
IgA	−9 (−304–177)	15 (−39–234)	NS

[a] The antibody activity in the sample obtained at the time of diagnosis was subtracted from that of the two week follow-up. The change is given as the median difference in antibody activity in each patient group.
[b] See footnote Table 1.
[c] P value for the difference between the non-pregnant and the pregnant group (Mann-Whitney U test; significance <0.05). NS is not significant.

Urinary antibody response to acute pyelonephritis in non-pregnant and pregnant women

Paired urine samples were obtained from 48 non-pregnant women at the time of diagnosis and after a two week follow-up. The IgG and IgA antibody activities in urine are shown for each patient in Figure 2. The median and range values of IgG and IgA

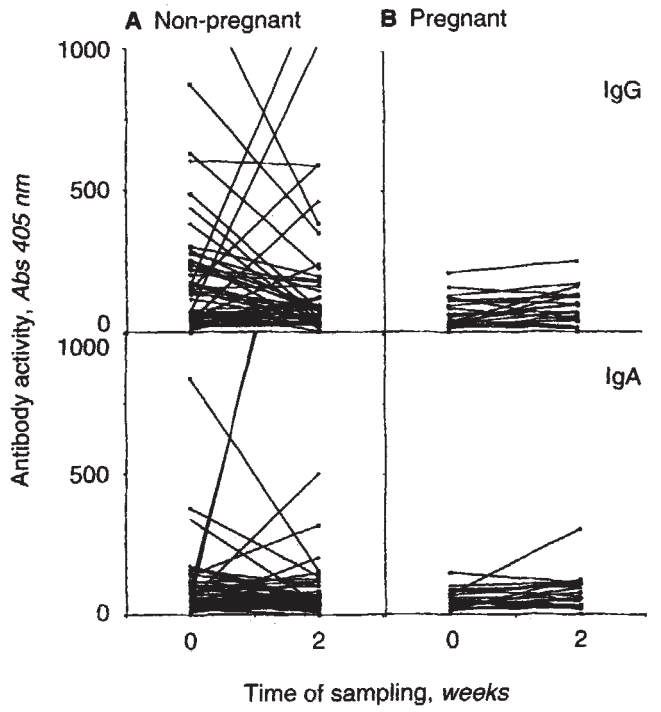

Fig. 2. *Urine antibody activity (IgG, IgA) at the time of diagnosis of acute pyelonephritis and at the 2 week follow-up.* Samples from the non-pregnant (**A**) and pregnant (**B**) women are shown separately. Samples obtained from each patient are connected with a solid line.

in urine at diagnosis and after two weeks are shown in Table 1. We compared the values at the time of diagnosis with those obtained at the two week follow-up in the individual patients using the Wilcoxon signed rank test. There was a significant decrease in the urinary IgG and IgA antibody activity in the non-pregnant group (Table 1).

Paired urine samples were obtained from 19 pregnant women at the time of diagnosis and after a two week follow-up. The IgG and IgA antibody activities in urine for the individual patients are shown Figure 2. The median and range IgG and IgA urinary antibody activities at diagnosis and after two weeks are shown in Table 1. There was a small increase in IgG and IgA antibody activity between diagnosis and the two week sample in the pregnant group.

The magnitude of the urinary antibody responses was compared between the non-pregnant and pregnant groups (Table 2). A significant difference was not found. However, there was a significant difference in urine antibody activity between the non-pregnant and pregnant groups at the time of diagnosis (IgG, $P = 0.03$; and IgA, $P = 0.019$) but not after two weeks (IgG, $P = 0.497$; and IgA, $P = 0.156$).

To exclude the confounding influence of age, we compared the urinary antibody responses in a subset of 13 non-pregnant women (19 to 37 years) to the 19 pregnant women (20 to 36 years). A difference in urinary antibody levels between the non-pregnant and pregnant groups was not observed either at diagnosis or at the two week follow-up (Table 2). Nor was there a significant difference in the change of antibody activity between the two sampling occasions.

Serum and urine IL-6 response to acute pyelonephritis in non-pregnant and pregnant women

The serum and urinary IL-6 response was analyzed in samples obtained at the time of diagnosis from 29 non-pregnant and 18 pregnant women with acute pyelonephritis. The IL-6 activity in serum and urine is shown in Figure 3. The responses in the non-pregnant and the pregnant groups were compared using the Mann-Whitney U test. There was a significant difference in the serum IL-6 response between the non-pregnant and the pregnant groups. The mean serum IL-6 levels were 50 U/ml for the non-pregnant and 5 U/ml for the pregnant group ($P < 0.05$). Serum IL-6 levels > 20 U/ml occurred in 14 of 28 non-pregnant compared to 1 of 12 pregnant women ($P < 0.001$).

There was a significant difference in the urine IL-6 response between the two groups. The non-pregnant women with acute pyelonephritis had a mean urinary IL-6 activity of 81 U/ml compared to 18 U/ml in the pregnant group ($P < 0.02$). Urinary IL-6 levels > 20 U/ml occurred in 25 of 29 non-pregnant compared to 3 of 18 of the pregnant women with acute pyelonephritis ($P < 0.02$).

Correlation of IL-6 with the acute phase response

The body temperature at the time of diagnosis was significantly higher in the non-pregnant group (median 39.5°C; range 38.0 to 40°C) than in the pregnant group (median 38.8°C; range 38.1 to 41°C; $P < 0.019$). Since IL-6 acts as an endogenous pyrogen, we analyzed the relationship between the serum IL-6 levels and the febrile response. There was no correlation between the temperature and serum IL-6 levels in the pyelonephritis group as a whole, or in the non-pregnant ($r = 0.2$, $P < 0.35$) or pregnant patients ($r = 0.3$, $P < 0.44$) with acute pyelonephritis.

The mean (± SD) CRP levels were significantly higher in the non-pregnant women with acute pyelonephritis (179 ± 125 mg/liter) compared to the pregnant group (101 ± 78 mg/liter; $P < 0.026$). IL-6 is an activator of the CRP synthesis in the liver. We therefore analyzed the relationship between IL-6 and CRP levels in the serum by linear regression. There was no correlation of the individual CRP and serum IL-6 levels within either the non-pregnant or pregnant groups ($r = 0$, $P < 0.9$). There was a highly significant correlation between the fever and CRP levels in the individual patients with acute pyelonephritis ($r = 0.5$, $P < 0.0001$).

Interleukin-6 and antibody responses

IL-6 stimulates B lymphocyte differentiation and antibody production. We used linear regression to analyze the possible association of IL-6 and antibody responses in individual patients. An association between the levels of IL-6 activity in serum and the change in serum IgG, IgM or IgA antibodies was not observed for the non-pregnant women with acute pyelonephritis ($r = 0.258$; $r = 0.059$; and $r = 0.019$, respectively). Similarly, there was no correlation of serum IL-6 levels with serum IgG, IgM or IgA antibodies in the pregnant patients ($r = 0.232$; $r = 0.027$; and $r = 0.188$, respectively). Regression analysis also showed there was no association between the urinary IgG and IgA antibodies and urinary IL-6 levels in both patient groups (IgG, $r = 0.019$ and IgA, $r = 0.033$ for the

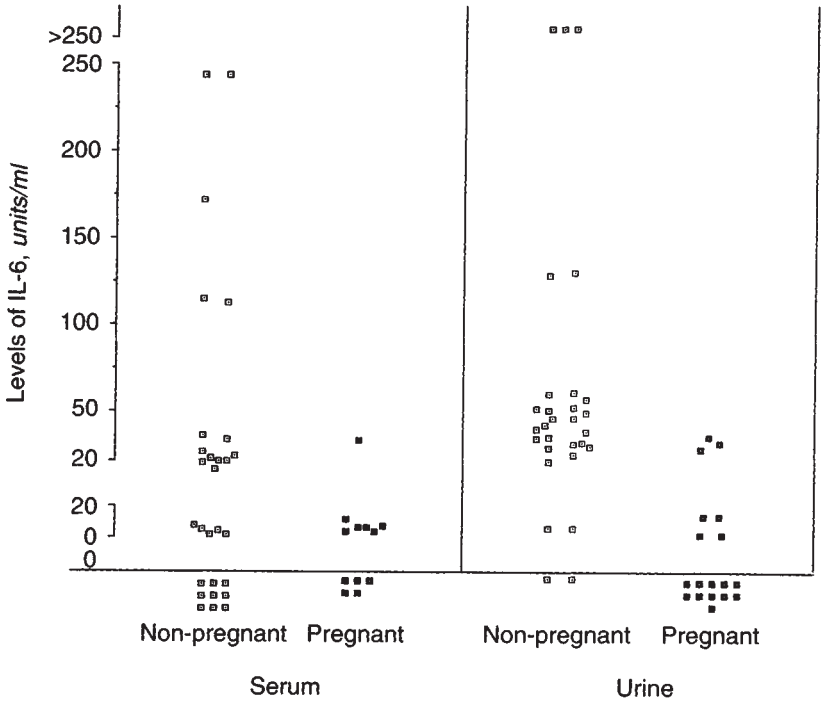

Fig. 3. *Urine and serum IL-6 activity in non-pregnant and pregnant women at the time of diagnosis of acute pyelonephritis.*

non-pregnant group; IgG, $r = 0.144$ and IgA, $r = 0.067$ for the pregnant group).

Discussion

This study demonstrated that pregnant women had reduced IL-6 and serum antibody responses to acute pyelonephritis compared to non-pregnant women. The serum IgG, IgA, and IgM antibody activity to *E.coli* antigens increased in the non-pregnant women during the two weeks following the diagnosis of acute pyelonephritis. In contrast, the increase in serum anti-*E.coli* antibody activity of the pregnant women was less pronounced. The serum and urinary IL-6 levels were elevated in the majority of the non-pregnant women with acute pyelonephritis, but low in the pregnant women at diagnosis. These results suggest that pregnancy is accompanied by an immunosuppression of the active cytokine and specific antibody responses to gram-negative UTI.

The main purpose of the study was to evaluate the immune response to acute pyelonephritis of individual patients, and the effect of pregnancy on this response. We obtained samples from each patient at the onset of infection and two weeks later. The response to infection was deduced from the difference in antibody activity between these samples. The antibody activity in serum and urine was determined using antigens prepared from the *E.coli* strain infecting each patient. The antigen extracts contained heat-stable lipopolysaccharides and proteins (including fimbrial proteins) from the infecting strain [26]. Broadly reactive antigen preparations were used to permit the detection of multiple antibody specificities. This study was not designed to analyze antibody responses to single antigen epitopes of the bacterial strains.

The antibody response to acute pyelonephritis has been the subject of numerous studies [5–9, 27]. The serum antibody response which occurred among the women with acute pyelonephritis in the present study was consistent with the findings in these previous studies. Vosti, Monto and Rantz analyzed anti-O antibodies in serum of women with acute pyelonephritis and acute cystitis [30]. A significant rise in serum antibodies occurred in 14 of 17 patients with acute pyelonephritis with the antibody response reaching maximal levels in the third to fourth week [30]. Andersen [27] and Jodal [31] followed the serum antibody response to acute pyelonephritis in children. A serum antibody response occurred in a majority of the children and was even used as a diagnostic tool in this patient group.

In contrast, the active immune response in pregnant women to UTI has not previously been examined. Previous studies of antibody responses in pregnancy have focused mainly on the pre-existing antibody levels rather than the active response to infection. The results of this study showed that pregnant women had a lower systemic antibody response to acute pyelonephritis than the non-pregnant group. This might reflect a reduced immune response or lower immunogenicity of the *E.coli* strains that infect pregnant women. The *E.coli* clones infecting pregnant women differ in terms of their O:K:H antigen combinations from the clones infecting non-pregnant women. There is, however, extensive overlap in individual antigens such as lipopolysaccharide [LPS] and the P-fimbriae. These antigens are known to be immunogenic and have been proposed to be immunodominant. It is unlikely that the whole pregnant group would be selectively infected by clones of low immunogenicity especially since their strains carry antigens known to be immunogenic in other individuals. We therefore consider it likely that the reduced host response in the pregnant group was caused by the immunosuppression of pregnancy.

The urinary antibody response to acute pyelonephritis has mainly been studied in children [5, 7, 8]. An increase in urinary

antibody activity occurred around 7 to 10 days after infection, and was of short duration [8]. The collection of urine samples in the present study was timed according to the information from the pediatric studies. However, the kinetics of the antibody response among the adult women differed from the pattern seen in children. A large fraction of the patients had elevated urinary antibodies at the time of diagnosis. The urinary antibody activity in those patients decreased rather than increased with time. This antibody response was not caused by earlier UTI episodes. Although several of the study patients had experienced prior UTI episodes, these were too far in the past to influence the urinary antibody levels at the time of enrollment in the present study.

Previous studies in adults have shown that IL-6 is secreted into the urine within a few hours of deliberate bacterial challenge, and that IL-6 is present in the urine at the time of diagnosis in patients with bacteriuria [10, 11]. In this study the pregnant women were found to have a reduced IL-6 response during UTI. Pregnancy is accompanied by a decrease in urinary osmolarity. It may be discussed if the apparent decrease in urinary IL-6 levels in the pregnant women was caused by the dilution factor rather than by a decreased production of IL-6. Two observations argue against this hypothesis. First, there was a concomitant decrease in serum IL-6 levels. Second, there was no decrease in the urinary antibody levels and no association of urinary antibodies with IL-6. This suggested that pregnant women have a reduced serum and urinary IL-6 responses to UTI.

IL-6 is an endogenous pyrogen, an activator of the acute-phase response and a stimulator of B lymphocyte differentiation, especially for the IgA committed mucosal B-cells [32–35]. Pregnant women have reduced levels of host response parameters like fever and CRP. The low serum levels of IL-6 might explain the reduced host responses in the pregnant group. We attempted to correlate IL-6 activity with the temperature, CRP and the IgA response of the individual patients. A significant correlation between these responses was not observed. This dilemma has previously been observed in adult patients with acute pyelonephritis where IL-6 levels did not correlate with the inflammatory response [11]. The role of IL-6 in the *in vivo* response therefore remains to be defined. The kinetics of the IL-6 response and the acute phase reactants may differ by some time factor which does not allow a correlation to be made.

The immunosuppression which occurs during pregnancy does not lead to a generalized susceptibility to infection. However, the rate of acquisition of bacteriuria seems to be increased during pregnancy and bacteria of reduced virulence have been shown to cause acute pyelonephritis in pregnant women [24, 36]. The tendency to develop acute pyelonephritis has been attributed to the hydrodynamic changes of the urinary tract which facilitate retrograde transport of bacteria to the kidneys [14]. The results of the present study demonstrated that the active immune response to UTI is impaired in pregnant women. This raises the question whether the reduced IL-6 and antibody responses also influence the susceptibility to acute pyelonephritis in pregnancy.

The contribution of specific immunity and mucosal antibodies to the resistance against UTI is not well understood. The main evidence favoring a role of humoral immunity has been obtained from studies in animal models. Hyperimmunization protects against subsequent bacterial challenge, however, it is not clear to what extent this protection is explained by antibodies [37–39]. Several observations contradict a role of specific immunity for the natural resistance against UTI in patients or animals who have not been hyperimmunized. The frequency of UTI is not increased in patients with antibody deficiencies like hypogammaglobulinemia. Furthermore, immunodeficient mice have intact resistance to experimental UTI [40, 41]. It is therefore difficult to argue that the reduced antibody response of the pregnant women is the cause of their susceptibility to symptomatic UTI.

The resistance to experimental UTI is influenced by the inflammatory response to infection [37–42]. *E.coli* fail to activate an inflammatory response in mice of the Lps^d genotype [40, 41]. These mice are highly susceptible to *E.coli* UTI. Furthermore, animals treated with anti-inflammatory agents have impaired ability to clear experimental UTI [42]. The low IL-6 response may indicate that pregnant women have a generally reduced level of mucosal inflammation. This may include factors that are crucial for the elimination of infection.

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